

Nesting of the Large-billed Reed Warbler *Acrocephalus orinus*: a preliminary report

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Large-billed Reed Warbler *Acrocephalus orinus* has a limited breeding distribution. It is known to inhabit valleys of the Panj river and its tributaries in Gorny Barakhshan Autonomous Republic (Tajikistan) and Badakhshan province (Afghanistan). Here we give descriptions of nests and eggs of this species based on 18 fresh nests found in Panj and Ghund valleys (Tajikistan) in 2011. Unlike the closely related species *A. dumetorum* and *A. scirpaceus*, Large-billed Reed Warbler has nests built with a layer of wool and seed tufts. Nests are placed on twigs of sea-buckthorn, willow and other bushes, herbs and reed stems over dry soil. Large-billed Reed Warbler clutch size is relatively small (on average, 3.77 ± 0.83 eggs ($n = 13$)). The ground colour of eggs is usually white, not bluish, greenish or rosy as in the related species.

INTRODUCTION

Until recently Large-billed Reed Warbler *Acrocephalus orinus* remained one of the least studied bird species of the Palearctic fauna. A. O. Hume discovered the first specimen on 11 November 1867 in the Sutlej valley, Himachal Pradesh, India (Hume 1869, 1870). He described the bird as *Phyllopneuste macrorhynchus*, and later referred to it as *A. macrorhynchus*. Oberholser (1905) changed the name to *A. orinus*.

The taxonomic status of this form remained uncertain until the beginning of the twenty-first century when Bensch & Pearson (2002) studied the type specimen in detail, including sequencing of mitochondrial and nuclear DNA. This study confirmed the specific status of Large-billed Reed Warbler, although some doubts remained (McCarthy 2006) until the moment when P. D. Round caught a live bird near Bangkok, Thailand (Round *et al.* 2007). Further studies of museum collections and searches for living individuals helped to elucidate possible breeding, moulting and wintering areas (Svensson *et al.* 2008, 2010, Timmins *et al.* 2009, Koblik *et al.* 2010, 2011).

In 2009, a bird feeding fledglings was caught in south-east Tajikistan, not far from the border with Afghanistan, in the Shakhdara river valley (Ayé *et al.* 2010). Museum specimens in the Zoological Museum of Moscow University (Moscow, Russia) and the Institute of Zoology and Parasitology (Dushanbe, Tajikistan) reveal that previous records of Blyth's Reed Warbler *Acrocephalus dumetorum* breeding in the Vanj and Ghund river valleys in fact refer to Large-billed Reed Warbler (Kvartalnov *et al.* 2011a,b, Kvartalnov & Garibmamadov 2012). Although fledglings were recorded in 1961 by A. V. Popov in the Vanj valley, near the village of Ghijovast (Abdusalyamov 1973, Kvartalnov *et al.* 2011b) and in 2009 by R. Ayé and colleagues (Ayé *et al.* 2010), and birds collecting nest material were observed in 1976 by V. V. Kashinin in the lower Ghund valley, near the village of Barsem (unpublished manuscript – see Kvartalnov *et al.* 2011a), no nests of this species have ever been described.

The mystery of the Large-billed Reed Warbler could have been solved in the mid-twentieth century. When in 1937 A. B. Kistyakovsky took part in a Pamir expedition, he found that reed warblers in the south-western Tajik Pamir mountains (in the environs of the town of Khorog) were not typical Blyth's Reed Warblers. He therefore prepared a description of a new *A. dumetorum* subspecies, but his manuscript and the intended type series were destroyed in a fire together with all zoological collections in Kiev University during the German occupation in the Second World War (Nowak 2001). Kistyakovsky (1950) wrote his opinion of the systematic position of this form, which he thought to be

endemic to Gorny Badakhshan, Tajikistan. Other naturalists who had visited the Pamir mountains in the twentieth century did not distinguish those birds from typical Blyth's Reed Warblers.

In 2010–2011 we studied spring migration of Blyth's Reed Warbler and breeding biology of Large-billed Reed Warbler in Tajikistan. Blyth's Reed Warbler was found to be common during spring passage in the vicinity of Dushanbe and in the south-west part of the republic, but we failed to prove its breeding in Tajikistan, although this was suspected by Abdusalyamov (1973), Portenko & Stübs (1976) and other ornithologists. There is no doubt that Blyth's Reed Warbler is a transient in all regions to the south of Almaty in south-east Kazakhstan, and that all nests found there that had been attributed to that species belong to others, including Paddyfield Warbler *Acrocephalus agricola* and Sykes's Warbler *Hippolais rama* (Ivanitskii *et al.* 2012).

Data about phenology, breeding biology, social behaviour, acoustics and morphometry of the Large-billed Reed Warbler collected in 2011 are presented in this article with additional data from 2012. This information is to help other ornithologists to search for and distinguish nests of Large-billed Reed Warbler.

MATERIALS AND METHODS

The main field observations were conducted in the Panj valley near the village of Zumudg, Ishkashim region, Gorny Badakhshan Autonomous Region, Tajikistan (36.917°N 72.183°E) between 10 June and 11 July 2011. Additional data were collected in the Apharv forest area in the Panj valley (36.800°N 71.550°E) and near the village of Langar in the lower Pamir river valley (37.033°N 72.667°E). AA inspected riverside forests in the Ghund valley near the villages of Charthem (37.717°N 72.167°E), Vuzh (37.717°N 71.933°E) and Dehmiyona (37.700°N 71.917°E) (Figure 1). From 23 May to 23 July 2012 PK and colleagues studied breeding biology and social behaviour of Large-billed Reed Warbler near the village of Dehmiyona; the resulting data are not included here.

Nine adult Large-billed Reed Warblers were caught in mist-nets and traps at nests near Zumudg (Plate 1). The birds were identified by measurements of bills, wings, tails and legs according to Svensson *et al.* (2010), Koblik *et al.* (2010) and from our experience of working with series of Large-billed and Blyth's Reed Warblers in collections of the Zoological Museum of Moscow University (Ivanitskii *et al.* 2012). Adults were marked with metal and colour rings and by grease paint colouring on breast and head for individual identification (a harmless method that we used previously with other warbler species). Blood samples were taken



Figure 1. A map of localities of some historical and recent observations of Large-billed Reed Warbler *A. orinus* in Tajikistan.



Figure 2. The relationships between Large-billed Reed Warbler and closely related species of the genus *Acrocephalus* based on NJ and MP analyses. Genbank accession numbers for Eurasian Reed Warbler *A. scirpaceus*, Marsh Warbler *A. palustris* etc. are given in the text.

from all caught adult birds and eight nestlings (also marked with metal rings). Specific identification was supported by analysis of mt DNA (Figure 2).

Total DNA was extracted from dried blood samples using the standard protocol of proteinase K digestion, phenol–chloroform deproteinisation and isopropanol precipitation (Sambrook *et al.*

1989). Mitochondrial DNA sequences were obtained from five Large-billed Reed Warblers caught in 2011 near Zumudg. The partial cytochrome *b* gene (207 bp) was amplified in one polymerase chain reaction (PCR) with the forward/reverse primer combination L14841/H15149 (Kocher *et al.* 1989). Typical conditions for *cytb* amplification included initial denaturation at



Plate 1. Panj river valley near Zumudg village, 25 June 2011.

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94°C for 3 min, 35 cycles of 94°C for 30 s, annealing at 51°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min and an indefinite hold at 4°C. PCR products were visualised on 1% agarose gel and then purified using DEAE (Whatman) or NH₄EtOH. Approximately 10–50 ng of the purified PCR product were used for sequencing with each primer by the autosequencing system ABI 3100-Avant in conjunction with ABI PRISM®BigDye™ Terminator, version 3.1.

Cytb sequences were aligned by eye using BioEdit 7.0. The final alignment of the mitochondrial region included 207 bp, of which 54 sites were variable and 40 sites were parsimony-informative. For the analysis we also used GenBank data (*A. dumetorum*, *A. orinus*, *A. agricola*, Eurasian Reed Warbler *A. scirpaceus*, Marsh Warbler *A. palustris* and Blunt-winged Warbler *A. concinens*). Phylogenetic neighbour-joining (NJ) and maximum parsimony (MP) analyses were performed using MEGA 4.0.0.4083. The NJ tree was reconstructed using the uncorrected p-distance. Unweighted MP analysis was performed using heuristic search starting with stepwise addition trees (random addition sequence, 10,000 replicates). To assess clade stability in the MP and NJ trees, 1,000 bootstrap pseudoreplicates were analysed.

GenBank accession numbers of obtained sequences are JQ651380–JQ651384. Genbank accession numbers of other sequences used in this work comprise: *A. agricola* AJ004245–AJ004248, AJ004330, AJ004331, AJ004775, AJ004776, FJ883021, Y15694; *A. concinens* AJ004260–AJ004262, FJ883027; *A. dumetorum*: AJ004263, AJ004264, AJ004336–AJ004340, AJ004773, FJ883028; *A. orinus*: DQ681065, GU247949–GU247958, HM352785, HM352786, HM352789; *A. palustris*: AJ004293, AJ004294, AJ004344, AJ004345, AJ004774, EU861031, FJ883036; *A. scirpaceus*: AJ004301–AJ004304, AJ004771, AJ004772, AM889139, FJ883039, NC 010227, Z73483.

The identification of uncaught birds was based on characteristic songs (Timmins *et al.* 2010, Ivanitskii *et al.* 2012). Recordings of songs of five marked males proved that the song described by Timmins *et al.* (2010) belongs to the Large-billed Reed Warbler (Ivanitskii *et al.* 2012). Nests that AA found in the Ghund valley were identified by comparison with known Large-billed Reed Warbler nests from the Panj valley. For comparison we also used unpublished data from 51 Blyth's Reed Warbler nests found and described by PK in 2007–2009 in the Kostroma region, Russia.

RESULTS AND DISCUSSION

The Large-billed Reed Warbler is a common species in suitable habitat in the Panj, Ghund and lower Pamir valleys. We found nests near the villages of Zumudg, Charthem, Vuzh and Dehmiyona, observed actively singing males in the Apharv forest area, and saw singing males and territorial pairs at the village of Langar (Figure 1). The birds inhabited thickets of sea-buckthorn *Hippophae ramnoides* and willow *Salix turanica*, *S. shugnanica* and *S. wilhemsiana* intertwined with clematis *Clematis hilariae*, with sparse ground cover of liquorice *Glycyrrhiza uralensis*, reed *Phragmites australis* and other species. Other bird species observed in the same habitat of Panj valley include Hume's Lesser Whitethroat *Sylvia althaea*, Mountain Chiffchaff *Phylloscopus sindianus*, Common Rosefinch *Carpodacus erythrinus*, Cetti's Bush Warbler *Cettia cetti*, Common Nightingale *Luscinia megarhynchos*, Bluethroat *Luscinia svecica*, Black-billed Magpie *Pica pica*, Isabelline Shrike *Lanius isabellinus phoenicuroides* and Citrine Wagtail *Motacilla citreola calcarata*. The only other *Acrocephalus* warbler recorded around Zumudg during our observations was Clamorous Reed Warbler *A. stentoreus* (a single transient bird). According to observations of PK in 2012, in the Ghund valley Large-billed Reed Warblers also breed in *S. turanica*, wild rose *Rosa beggerana* and honeysuckle *Lonicera stenantha* thickets with *Astragalus longistipitatus*, *Potamogeton cariatum* and other herbs along canals among crop fields on alluvial fans.

Most Large-billed Reed Warblers were found near river banks, canals or other wet localities. The birds breed in monogamous pairs, although attempted extra-pair copulations by at least three paired and two unmated territorial males were observed. We described 15 nests and one abandoned construction built in June and July 2011 in the Panj valley (Plates 2, 3), plus seven remains of nests built in 2009–2010 in the same area, and three recent nests in the Ghund valley in 2011. Thirteen nests had complete clutches. Nests are built by females (based on observations of building of nine nests in 2011, including two nests observed from the first day of construction). Most were in sea-buckthorn thickets, but one was in a willow bush. Nearly all nests were placed over dry soil, except one that was built on a branch over a canal temporarily filled by water.

Large-billed Reed Warblers attached nests to sea-buckthorn twigs at forks (six nests), stems of *Artemisia* (three nests), liquorice

stems (three nests), thin willow stems (two nests), willow twigs at forks (two nests), twigs of clematis at forks (two nests), reed stems (one nest), reed stems and willow twigs (one nest), reed and liquorice stems (one nest), liquorice stems and willow twigs (one nest), sea-buckthorn and willow twigs (one nest), reed, liquorice stems and a sea-buckthorn twig (one nest).

Females began nest construction with a platform of dry plant debris, but from the first day they braided vertical supports (stems, etc.) with plant fibres. Nests were fastened to stems more firmly than the nests of Blyth's Reed Warbler. The principal material consists of bast and bark fibres of clematis, willow, liquorice and *Artemisia*, fibres of reed sheath, dry leaves, stems and ears of grasses, goat wool and clematis seed tufts. Bast strips also form an outer covering that disguises the nest in thickets. An inner part of the structural layer is made with wool and seed tufts, and rarely includes bird feathers. Nests of Blyth's Reed Warbler usually lack such soft materials (Plate 5). The upper edges of Large-billed Reed Warbler

nest cups are made usually with ears of grasses. Nests are lined with clematis bast fibres or (rarely) with thin dry grass stems, with the addition of mammal hairs.

Nests found near Zumudg ($n = 15$) had the following measurements (average and standard deviation): outer diameter 81.7 ± 8.0 mm; height 68.6 ± 9.4 mm; inner diameter 54.8 ± 2.0 mm; depth 45.1 ± 3.0 mm. The height of nests above ground or water was 30–168 cm (average 82.1 cm). Nests inspected in the Ghund valley in 2011 were placed at 110–210 cm above ground.

Most full clutches in the Panj valley were of four eggs ($n = 7$), rarely two ($n = 1$), three ($n = 3$) or five eggs ($n = 2$); on average, 3.77 ± 0.83 ($n = 13$), including a replacement clutch that contained three eggs (the first clutch consisted of four eggs). We also found a nest with three nestlings. Nests found in Ghund valley had three (two nests) and four (one nest) eggs. The usual clutch size of the Blyth's Reed Warbler in Kostroma region was 5–6 eggs, rarely four eggs, on average ($n = 37$) 5.53 ± 0.56 eggs.

Plate 2. Nest 5-11 of the Large-billed Reed Warbler *A. orinus* with full clutch (two unhatched eggs from this nest are now held in the Natural History Museum, Tring, UK), 21 June 2011.



Plate 4. A clutch from nest 13-11 of the Large-billed Reed Warbler showing markings on eggs, 11 July 2011.



Plate 3. Nest 11-11 of the Large-billed Reed Warbler with full clutch (now held in the Zoological Museum of Moscow University), 9 July 2011.



Plate 5. A nest of Blyth's Reed Warbler *A. dumetorum* with full clutch, Kostroma region, Russia, 14 June 2007.



Clutches of Blyth's Reed Warbler from South Siberia had on average 4.8–5.2 eggs (Totunov 1981) or 5.1–5.76 eggs (Kuranov 2008) in different years.

Eggs of Large-billed Reed Warbler have a dirty-white (rarely creamy white or pure white) shell covered with small olive-brown superficial spots that usually (but not always) form a sparse cap or a ring at the larger end, and with more sparse dark-brown superficial specks which rarely (as opposed to eggs of Blyth's Reed Warbler) lay over larger spots (Plates 4 & 5). Sparse and small deep bluish spots also form a cap or a ring at the larger end. Some eggs are covered not only with sparse spots but also with dense small olive brown specks that can hide the basic shell colour. Fresh eggs are dull or with a weak gloss. Eggs of Blyth's Reed Warbler are greenish or rosy, usually with no white background (Chernyshov 1998; our data). Measurements of eggs (in mm; n = 58): 17.72 ± 0.55 (16.8–19.1) \times 13.14 ± 0.31 (12.3–13.8). Egg weight (n = 48): 1.51 ± 0.15 (1.13–1.76) g. One Large-billed Reed Warbler nest with a

complete clutch (three eggs) is now deposited in the Zoological Museum of Moscow University (ZMMU Q-8036); two unhatched eggs were sent to the Natural History Museum, Tring, UK (NHMUK E/2012.5.1).

Eurasian Reed Warbler has been found in Gorny Badakhshan and nearby Afghanistan, although it is not known to breed there (Abdusalyamov 1973, Timmins *et al.* 2010). It has nests without the layer built with wool and seed tufts (Kvartalnov *et al.* 2006), and eggs with bluish or olive-greenish basic shell colour, not white (Nikiforov *et al.* 1989).

Only female Large-billed Reed Warblers have brood-patches, but both partners incubate eggs and provide food to nestlings and fledglings (Plates 6, 7, 8).

Large-billed Reed Warblers in the Panj valley have a relatively wide range in dates of arrival on the breeding grounds (compared with Mountain Chiffchaff and other passerines inhabiting the Pamir Mountains: our observations). The nest found on 5 July with young birds ready to fledge must have been built during the first days of June. Most other nests near Zumudg were built after 10 June. Several new birds reached the breeding grounds at the end of June and the beginning of July. The latest of the first clutches found near Zumudg was finished on 7 July. On the last day of investigations (11 July) we observed a female in the territory of a male which was singing from 9 July. Of two nests built for replacement clutches, one was still empty on 11 July. In the lower Pamir river valley on 2 July we found several actively singing bachelor males, newly formed pairs and apparently non-territorial birds. AA found nests with clutches on 23 June, 16 July (nestlings hatched 17 July) and 17 July in the Ghund valley. V. V. Kashinin in the lower Ghund valley (37.550°N 71.733°E) observed the peak of nest building to be in mid-June (Kvartalnov *et al.* 2011b). A. V. Popov saw fledglings on 22 June 1961 in the Vanj valley (38.550°N 71.733°E) (Abdusalyamov 1973, Kvartalnov *et al.* 2011b); fledglings possibly of this species were recorded by A. V. Popov in the Shakh dara valley on 26 July (birds were not collected, and the year is unknown) (Abdusalyamov 1973). Ayé *et al.* (2010) saw fledglings near the Shakh dara River on 19 July 2009. According to museum collections (Koblik *et al.* 2010, 2011) and our observations in 2012, Large-billed Reed Warblers reach breeding grounds in Gorny Badakhshan in the final third of May. AA saw the last birds that he thought to be this species in the first ten days of September in the Panj valley, but this needs to be confirmed by mist-netted birds.

Plate 6. A newly-hatched nestling of Large-billed Reed Warbler from nest 5-11, 3 July 2011.



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Plate 7. Female Large-billed Reed Warbler feeding nestlings, nest 1-11, 10 July 2011.



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Plate 8. A young Large-billed Reed Warbler ready to fledge, nest 12-11, 5 July 2011.



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